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Dimethylmercury Production in Freshwater Sediments

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DIMETHYLMERCURY PRODUCTION IN FRESHWATER SEDIMENTS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY David Kelly ENTITLED, Dimethylmercury Production in Freshwater Sediments, BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Kelly, David C. M.S., Department of Earth & Environmental Sciences, Wright State University, 2010. Dimethylmercury Production in Freshwater Sediments.

Dimethylmercury (DMHg) has been examined almost exclusively in marine environments to date, plays an uncertain role in the global cycling of Hg, and is produced by a currently unknown mechanism in natural systems. We examined DMHg production in microcosms containing 100 cm^3 of freshwater sediment sampled from 3 wetlands and a lake near Dayton, Ohio. DMHg was produced from all sediments analyzed, and found that production is increased significantly by the addition of inorganic Hg from ~0.1pM to ~10pM, its production is unaffected by autoclaving the sediment, and organic carbon additions had no discernible effect on production. Its total concentrations are most likely the result of an interaction between methylating and demethylating processes. These results suggest that DMHg production may be pervasive among freshwater sediments.

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INTRODUCTION

Mercury (Hg) is a trace metal that is found throughout the natural environment, has no beneficial biological function, has a complex biogeochemical cycle (Fitzgerald and Lamborg, 2003), and is toxicologically significant as monomethylmercury (MMHg) (Gochfeld *et al.*, 2003). Loadings of Hg to the environment have been increasing, even in remote locations, due to the burning of fossil fuels and through industrial processes that use Hg (Pacyna and Pacyna, 2002; Pacyna et al., 2006). Greater loadings of Hg, mostly through atmospheric deposition, are hypothesized to result in increased levels of MMHg in biota (Hammerschmidt and Fitzgerald, 2005; Hammerschmidt and Fitzgerald, 2006; Orihel et al., 2007).

Dimethylmercury ($(CH_3)_2Hg$, DMHg) is a volatile and extremely toxic (WHO, 1990) form of mercury that has been examined almost exclusively in marine environments, where it has been found to be the main form of methylated Hg in the deep ocean (Fitzgerald *et al.,* 2007). In non-marine environments, DMHg has been oberved in anoxic waters of Lake Onondaga (Bloom and Effler, 1990), mangrove and saltmarsh sediments (Quevauviller *et al*., 1992; Weber *et al*., 1998), river floodplain soil (Wallschläger *et al*., 1995), and in gas from landfills (Lindberg et al., 2005). The lack of information on DMHg in freshwater sediments is due in part to freshwater production being considered negligible (Bloom and Effler, 1990; Vandal *et al.*, 1991).

The exact mechanism(s) of DMHg production and its overall role in the global cycle of Hg, especially in freshwater environments are not known; however, DMHg is

hypothesized to be produced mostly through microbial processes involving a mechanism similar to MMHg production (Choi et al., 1994). It is unclear if DMHg is either a precursor to MMHg formation involving the degradation of DMHg into MMHg. DMHg could also form from remethylation of MMHg, which is either formed accidentally (Choi *et al*., 1994) or purposefully (Robinson and Tuovinen, 1984). Craig and Bartlett (1978) proposed DMHg was formed from a disproportionation reaction involving MMHg and S^{2-}, but this was a purely chemical reaction and would not easily account for DMHg found in aerobic environments (Mason et al., 1998) or those with low levels of free S^{2-} (Lamborg et al., 2008).

If DMHg were formed from MMHg, its production would depend heavily on processes that produce MMHg. MMHg in the environment is thought to be produced from the activity of sulfate- (Gilmour et al. 1992) and iron-reducing bacteria (Fleming et al., 2006). However, abiotic production from reactions of inorganic Hg (Hg^{2+}) with organic material or methylated metals are likely to occur as well (Weber, 1993; Bloom et al., 1997; Falter et al., 1999). Once produced, MMHg can be degraded by microorganisms present in the sediment (Summers, 1986), and the pool of Hg available for methylation can be diminished by reduction to, and eventual loss of, Hg^0 via biotic or abiotic processes (Robinson and Tuovinen, 1984). Net methylation of Hg to DMHg relies on the interaction of these methylation and demethylation processes and may be the result of an interaction between biotic and abiotic factors.

Given the lack of information on the mechanism(s) of DMHg formation, its production in freshwater environments, and its possible relationship to MMHg, we investigated DMHg production in bench-scale microcosms with freshwater sediments to

examine the factors that may influence its production. We analyzed sediments from several freshwater environments in Ohio including Siebenthaler Fen, Valle Green wetland, a constructed wetland on the Wright Patterson Air Force Base (all in Greene County) as well as Crystal Lake (Clark County). Results of our tests show that each sediment produced DMHg under ambient conditions and that the rate of DMHg production was limited by Hg availability.

METHODOLOGY

Microcosms

Site Comparison

The sediments (described in the previous section) were obtained from a depth of approximately 0.3m below the soil surface by removal with a shovel in order to avoid large pieces of organic matter like roots and leaves. Sediments were seived to remove any particles greater than $1/8^{th}$ of an inch, and $100cm^3$ aliquots (~160g) were placed inside the experimental microcosms, which were 1L glass flasks sealed with a modified silicon stopper and kept in an anoxic glovebox (Fig. 6). The microcosms were amended with 600mL of basal media (Fogel et al. 1986) and sediment, sealed, and then incubated before each sampling. Aqueous samples were withdrawn with a syringe though a sampling port. A portion was passed through a syringe filter (0.2μm) prior to analysis for Fe^{2+}, total S^{2-}, SO_4^{2-}, and NO_3^-. Unfiltered water was analyzed for pH and dissolved gases (O_2, CH_4 and CO_2). DMHg was purged from solution promptly after water sampling with N_2 gas and collected on a Tenax trap attached to a vent on the silicone stopper. The microcosms were sampled once a week for nine weeks.

Hg Limitation

To a separate batch of Siebenthaler Fen sediment, we investigated whether Hg(II) may limit sediment production of DMHg. Sediments in three microcosms were amended with 160μg of Hg(II), as $HgCl_2$ (nominal concentration ~1μg/g) whereas three others

were not treated (controls). Sediments were sampled every three to four days for 46 days. Water quality parameters and DMHg were determined as described above.

Abiotic vs. Biotic

We also attempted to examine whether the production of DMHg was by a abiotic or biological mechanism. A total of six microcosms were assembled, all with basal media and amended Hg(II) (nominal concentration ~1μg/g). To achieve an abiotic system, autoclaved sediment was used in three of the microcosms. Autoclaving of the sediment was done three times over the course of three days at 134ºC, and all other microcosm parts were autoclaved as well (flask, syringe, microcosm cap).

Effect of Organic Carbon

We also attempted to examine the effect that organic carbon would have on biotic and abiotic systems. Microcosms were assembled as described in the previous section (Abiotic vs. Biotic), however both sets of microcosms was amended with sodium acetate.

Analyses.

The aqueous samples from the microcosms were analyzed for Fe^{2+}, total S^{2-} (and periodically free S^{2-}), SO_4^{2-}, NO_3^-, DIC, CH_4, DO, pH and acetate by removal of liquid sample from the microcosm through the sampling port. Samples were filtered through a 0.22 μm, 25 mm dia. syringe filter (Restek, Bellefonte, PA) except for pH and dissolved gas analysis. The concentration of Fe^{2+} and total sulfide was determined by spectrophotometry using the phenanthroline and methylene blue methods, respectively (Lambda 45 UV/Vis, Perkin Elmer; Waltham, MA; Standard Methods, 1998, Appendix V; Standard Methods, 1998, Appendix VI). Sulfate, nitrate and acetate were analyzed by ion chromatography (Dionex Ion Chromatograph, DX 2500, Sunnyvale, CA; Appendix

IV). The pH was determined using a pH meter (AP10 pH/mV Meter, Denver Instruments, Bohemia, NY). Dissolved gas analysis was accomplished by removing 4.5mL of microcosm fluid into a gastight 9mL vial, which was allowed to equilibrate and then the headspace was sampled to determine the concentration inside of the microcosm using the approach of Burris et al., 1996. Aqueous concentrations were determined determining the partial pressure of the gas of interest and using the Gas Law and Henry's Law to determine concentration. DIC and DO were analyzed by a Hewlett Packard 5890 Series GC system with a thermal conductivity detector and were separated on a packed column (Shin Carbon 100/120, 2m x 1mm; Restek, Bellefonte, PA; Appendix XII). Methane was analyzed by a Hewlett Packard 6890 series GC system with flame ionization detection (FID), separated on a capillary column (GasPro, 30m x 0.32mm; J&W Scientific) connected to an FID (Appendix XI).

Hg Analysis.

DMHg was determined by isothermal gas chromatography cold vapor atomic fluorescence spectroscopy (GC-CVAFS) and confirmed by the retention time recorded on the analyzer (Horvat et al, 1993). Briefly, volatile DMHg was removed from the microcosms by purging with N_2 gas and collected on a Tenax trap (20:30 mesh) after being passed over a soda lime trap to remove excess moisture. The trap was then dried, and heated for detection on the GC column. After each of the experiments microcosm water and sediment samples were taken to analyze for MMHg and total Hg.

MMHg concentrations in water samples were determined by the procedure presented by Tseng et al, 2004. Briefly, a sample of diluted microcosm water is placed in a purging flask and its pH is adjusted with acetate buffer. A sodium tetraethylborate

solution is then added to volatilize the Hg and the solution is swirled to mix. After a reaction time, the solution is then purged to concentrate the volatile Hg on a Tenax trap. The trap is then dried for several minutes, to remove any residual condensation, before it is heated and the Hg passed through the GC column for detection.

MMHg in sediment was analyzed by a method of Horvat et al., 1993. THg in sediment was analyzed following the method from Fitzgerald et al., 2005. Briefly, the microcosms were disassembled and sediment samples were freeze dried. Samples were then digested by weighing out ~0.3g of soil into Teflon bombs and adding 5mL of a 3:2 mixture of HNO_3/HCl which was then microwave digested and allowed to sit overnight. The bombs were then diluted with reagent grade water and oxidized with BrCl. Samples were then analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS).

Data Treatment.

All experiments, except the freshwater sediment study, used triplicate microcosms using the same batch of sediment. Values obtained from analyses were averaged and the standard deviation was taken.

RESULTS AND DISCUSSION

Screening Level Test for DMHg Production. We examined a potential for DMHg production in each of the four test sediments with added Hg(II). Over a 9-week incubation period, net production of DMHg in individual sediment microcosms was found to be 7.92pmoles in Siebenthaler Fen, 6.64pmoles for WPAFB, 1.64pmoles for Valley Greene, and 1.78pmoles for Crystal Lake deposits. These preliminary results suggest that a potential for DMHg production exists among each of these deposits and, by extension, other freshwater sediments.

Role of Hg Availability

The roles of Hg availability and different terminal electron accepting processes were investigated with Siebenthaler Fen sediments. Fen sediment was selected for this test because it produced the most DMHg during our screening test. DMHg was produced in both Hg-amended (treatment) and unamended (control)sediments, although microcosms with added Hg produced orders of magnitude more DMHg. In both treatment and control sediments, DMHg was low or undetectable during the first two weeks of the test with a synchronous increase in production during the third week. At the end of the test, MMHg averages 0.9 ±0.1ng/g (control) and 90 ± 4ng/g dry weight (treatment) in sediment and 0.003 ± 0.005 ng/L (control) and 2100 ± 880 ng/L (treatment) in 0.2µm filtered water. Total Hg in 0.2µm filtered water averaged 47 ± 7ng/L (control) and 5200 ± 1800 ng/L (treatment). From this data it is clear that DMHg production is a function of the amount of Hg available for methylation, and these results

are consistent with findings of increased MMHg production due to increased Hg loadings (Hammerschmidt et al., 2006; Orihel et al., 2006).

There was significant variability in DMHg production among triplicate microcosms, which is most likely due to variations in either methylating and demethylating potential or differences in the microbial communities of the individual microcosms. There was little production of DMHg in both sets of microcosms before a large increase starting on day 21 (Fig.1). The lack of production at the start of the test could be due to the sediment being collected in the winter and an increased time required for microbial communities to grow, and there could be some inhibition from the Hg addition in the Hg amended microcosms as well. Alternatively, bottle effects may have limited microbial activity. This lag time would appear to indicate that DMHg is produced by a biological process in contrast to an abiotic one, which would be expected to more spontaneous. If iron- and sulfate-reducing bacteria were the main biological producers of DMHg, as is inferred for MMHg (Gilmour et al., 1992; Fleming et al., 2006), then we might expect to see an increase in DMHg production while those processes are occurring. However, the increase of DMHg production on day 21 is not consistent with observed patterns of measured Fe^{2+} and SO_4^{2-} in the microcosms, as sulfate in mesocosm water is nearly depleted before the onset of DMHg production (Fig. 2).

It could be the case that there was increased production during the period of Fe(III) and sulfate reduction but that these microbial groups also have a high demethylation potential for DMHg, and it could also be possible that the demethylation potential from other processes was higher in these sediments initially, before methylating processes were able to overcome them. Other processes, like acetogenesis could also be

more significant producers of DMHg in these sediments, as contributions from methanogens are unlikely due to our microcosms containing very low concentrations of methane. Inhibition of methylation by sulfide is unlikely as total sulfide was close to maximal concentrations of 0.00625mM before the significant increase of DMHg on day 21 (Fig. S4). The lack of production initially could also be due to the pathway of DMHg formation and the need for a pool of MMHg to be produced before remethylation can occur to produce DMHg.

The above analysis is generally consistent with our other experiments. DMHg production can be quite sporadic, and if it does appear to correspond to one terminal electron accepting process we can not say with certainty that the apparent process was responsible, as multiple microbial groups are active at any given time. It is also unknown how much each group can contribute to the DMHg pool, and as previously mentioned we were unable to determine the demethylating potential of our sediments, which may have a significant impact on final DMHg concentrations especially as they change over time presumably due to shifts in microbial activity.

Effect of Autoclaving

We also attempted to examine whether the production of DMHg was by an abiotic or biological mechanism by examining production from autoclaved and non-autoclaved sediments from Siebenthaler Fen. Sediments in three microcosms contained autoclaved sediment (treatment) and three contained non-autoclaved sediment (controls). We found that DMHg production was similar in both the treatments and controls and net production was 14.8 ± 6.6 pmoles in the control microcosms and 11.7 ± 2.9 pmoles in the treatments. The abiotic mechanism of DMHg production proposed by Craig and Bartlett,

1978, is unlikely to have occurred in our microcosms due to a lack of hydrogen sulfide caused by the presence of Fe^{2+} that would readily sequester it. The contribution from microorganisms to the DMHg pool in the treatment group is likely as sulfate decreased significantly in addition to Fe(II) increasing, which indicates iron- and sulfate-reducing bacterial activity.

DMHg in the third treatment microcosm (AF3) appears to be due to significant sulfate reduction that occurred before collection on day 5; however there was also iron reduction during this period (Fig. 5). There was still sulfate reduction occurring through day 9, however there was not significant DMHg production during this sampling interval. Iron reduction occurred through day 28, however DMHg production slowed considerably after day 5 except for two increases on day 19 and 26. As in our other studies, the demethylation potential of the sediment is not known and it may have a large influence on the DMHg production we saw. Recovery of DMHg diminished considerably after day 9 where demethylation activity could have increased due to microbial populations increasing or shifting.

Effect of Autoclaving and Organic Carbon Addition

Finally, we used Siebenthaler Fen sediments to investigate the effect that autoclaving and increased organic carbon would have on DMHg production. Three microcosms contained nonautoclaved sediment (controls) and three microcosms contained autoclaved sediment (treatment); all microcosms received acetate additions on day 0, 2, and 28 in order to prevent the concentration from reaching zero (Fig. S23). DMHg production was significantly higher in the treatment microcosms, which produced 41 ± 31 pmoles of DMHg compared to the 15.6 ± 11 pmoles produced in the controls.

The large standard deviations are due to two of the three microcosms in each group producing much more DMHg than the third. Acetate in the microcosms with autoclaved sediment increased substantially from day 2 through day 12 before leveling off, and there was also an increase in acetate levels from day 2 to day 5 in the control microcosms before it started to be consumed. The increase of acetate in the autoclaved sediments is most likely due to acetogenesis via fermentation although higher initial levels of acetate would be expected from autoclaving, which would release the organic carbon of the resident cells into the sediment, and it is possible that some acetate was leeching from the sediment.

Contamination or insufficient autoclaving of the treatment sediments is evident not only from the increase in acetate but also from the decrease in sulfate and rise of Fe^{2+} and methane, which was seen in the previous experiment as well. As in the previous study, there was no detectable sulfide most likely due to the presence of Fe^{2+}, and again there was significant DMHg production from the autoclaved sediments, and in general DMHg production is similar in both setups except for two samplilng dates where DMHg production was greatly increased (day 9 and 12; Fig. 5). Additionally, despite the apparent contamination of the autoclaved sediments, and high levels of acetate for microbial growth DMHg slows markedly towards the end of the experiment. This may be due to the speciation of Hg, which took on a form that would be unavailable for methylating processes or an increase in demethylation potential in the sediments. In the first (A1) and third (A3) control microcosms DMHg production was a factor of two higher than in the non-autoclaved sediments, however this was mostly due to a large

spike in DMHg production collected on day 9 in A1 and two large spikes collected on day 9 and 12 in A3.

Larger increases in DMHg in the control microcosms occur starting on day 9 after most sulfate and iron reduction has taken place. It could be that the remaining sulfate and iron reduction are directly responsible for this production, or that a change in the microbial communities causes a shift in the methylation and demethylation potentials which allows more DMHg to remain in the microcosm for collection. In the autoclaved microcosms significant DMHg production is mostly associated with sulfate consumption, however in the case of the third treatment microcosm (AA3) there was 24pM of DMHg produced before sulfate or iron reduction took place, and after these processes occurred production remained nearly the same with 25pM being produced. The acetogenesis occurring during this time could be responsible for the production as well, but we can not say how much each process contributed to the DMHg pool.

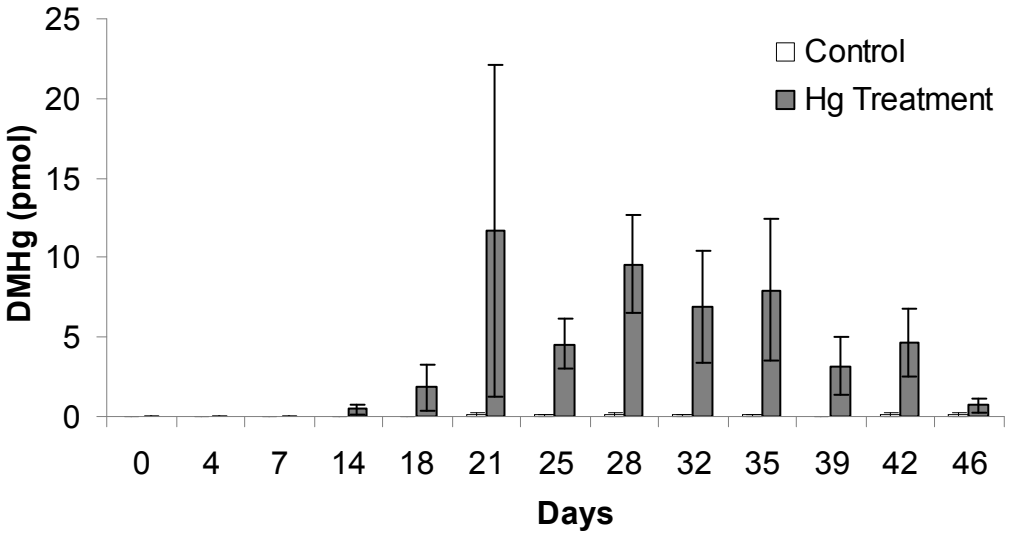

FIGURE 1. **DMHg production in Siebenthaler Fen sediments with and without added Hg (n=3 each; ± ISD)**

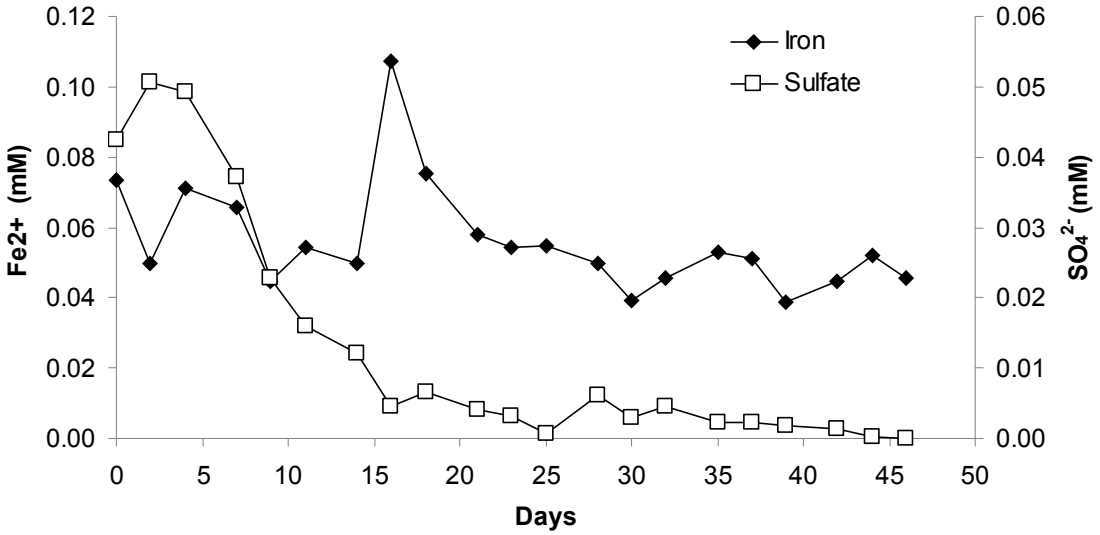

FIGURE 2. Fe(II) and sulfate concentration in the first treatment microcosm (H1) with Siebenthaler Fen sediment with added Hg (n=1; ± ISD)

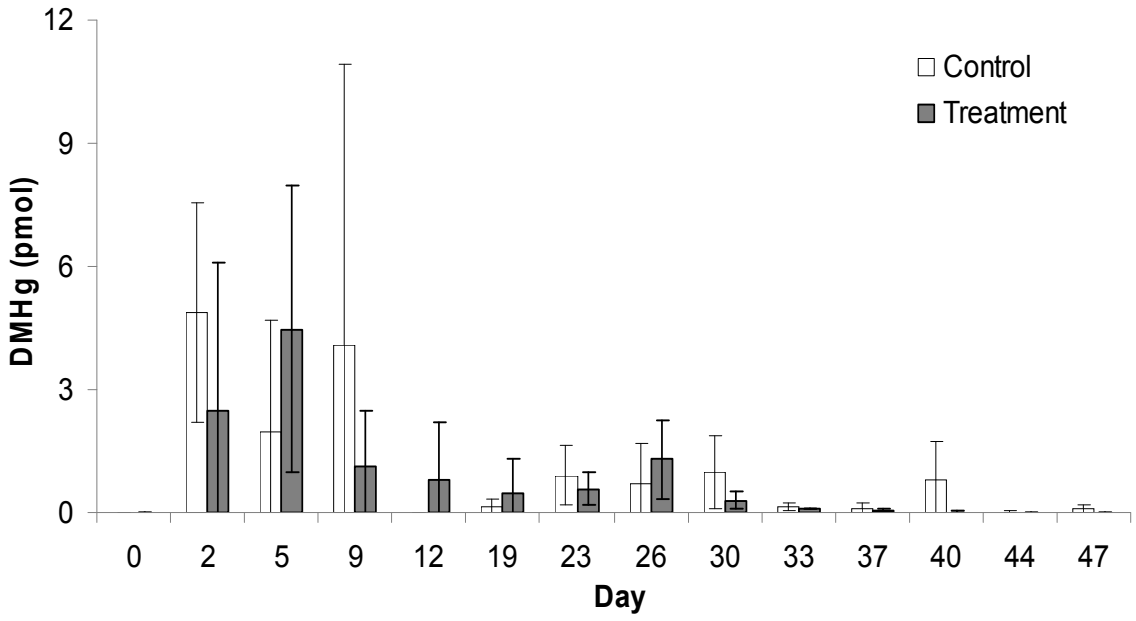

FIGURE 3. DMHg production in pM for the autoclaved and nonautoclaved Siebenthaler Fen sediments (n=3 each; ±ISD).

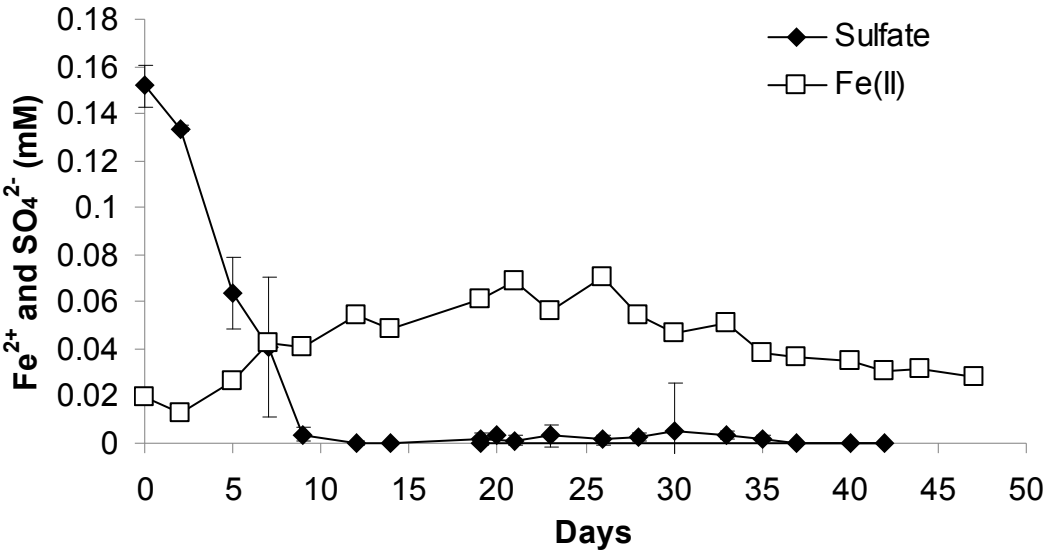

FIGURE 4. Fe(II) and sulfate concentration, in mM, for the AF3 microcosm with autoclaved Fen sediment and amended Hg.

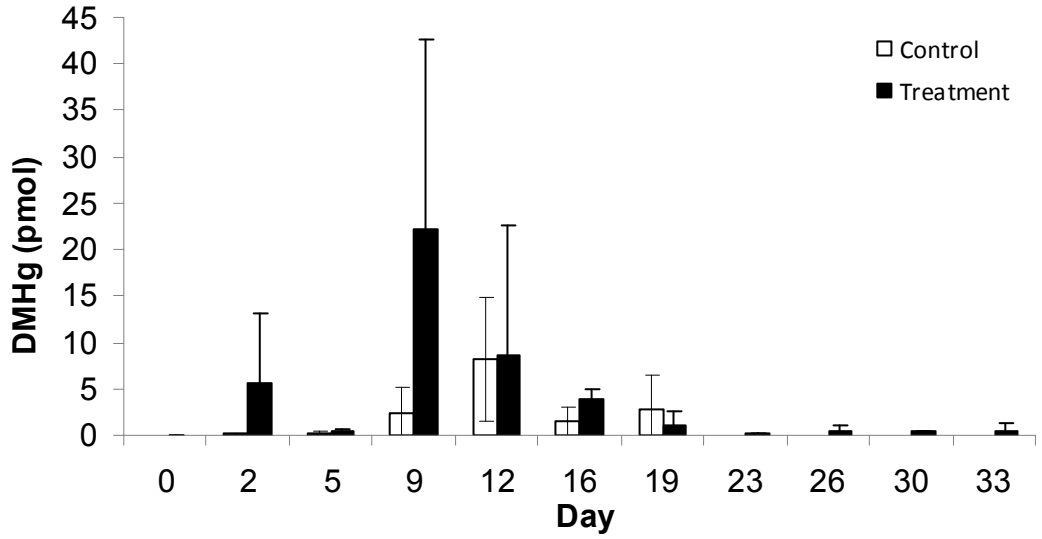

FIGURE 5. DMHg production in Siebenthaler Fen sediments with autoclaved and non-autoclaved sediments with acetate additions (n=3 each; ± ISD).

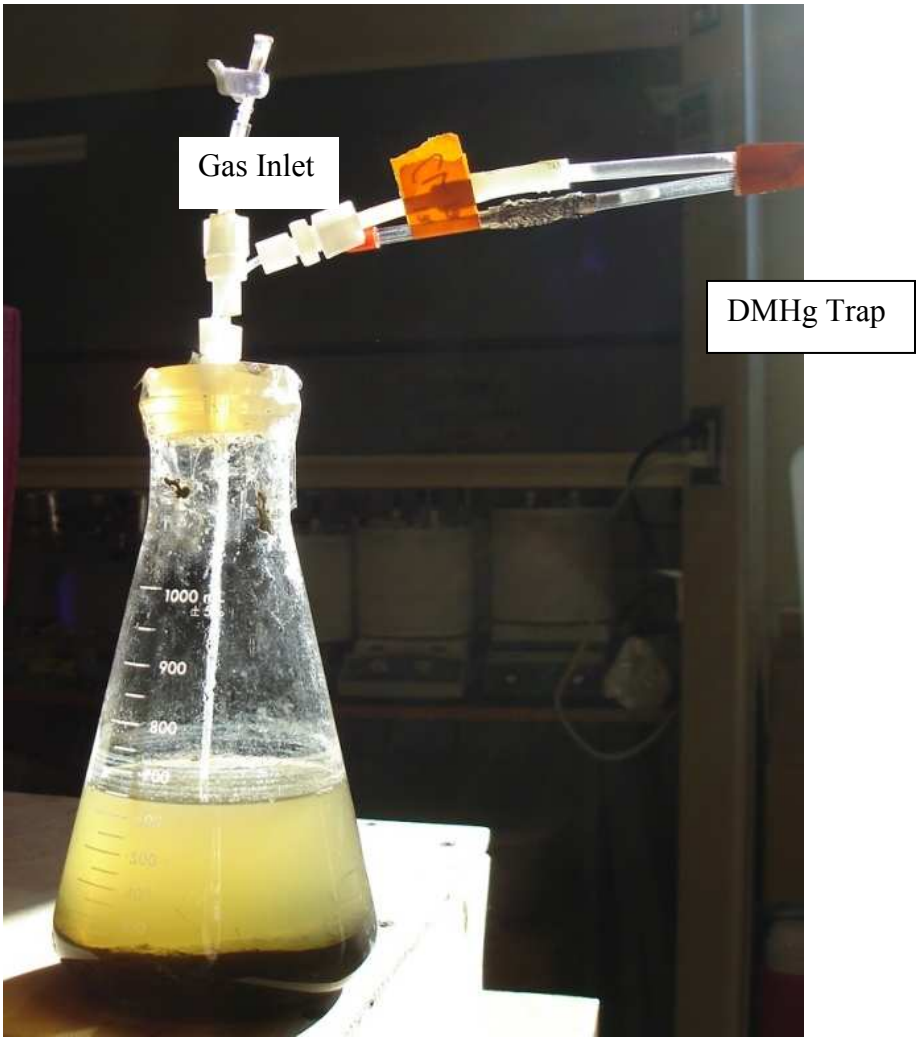

FIGURE 6. Experimental Microcosm. The gas inlet port is where nitrogen gas is used to purge the system of DMHg where it is collected on the Teflon DMHg trap. During experiments, there is 100cc of sediment and 600mL of basal media. The microcosms were covered with aluminum foil throughout the experiment to prevent the effect of light – which can demethylate Hg.

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WHO: Methylmercury environmental health criteria 101. International Programme on Chemical Safety, World Health Organization Geneva (France) 1990.

APPENDICES

Figures

Hg Addition Study

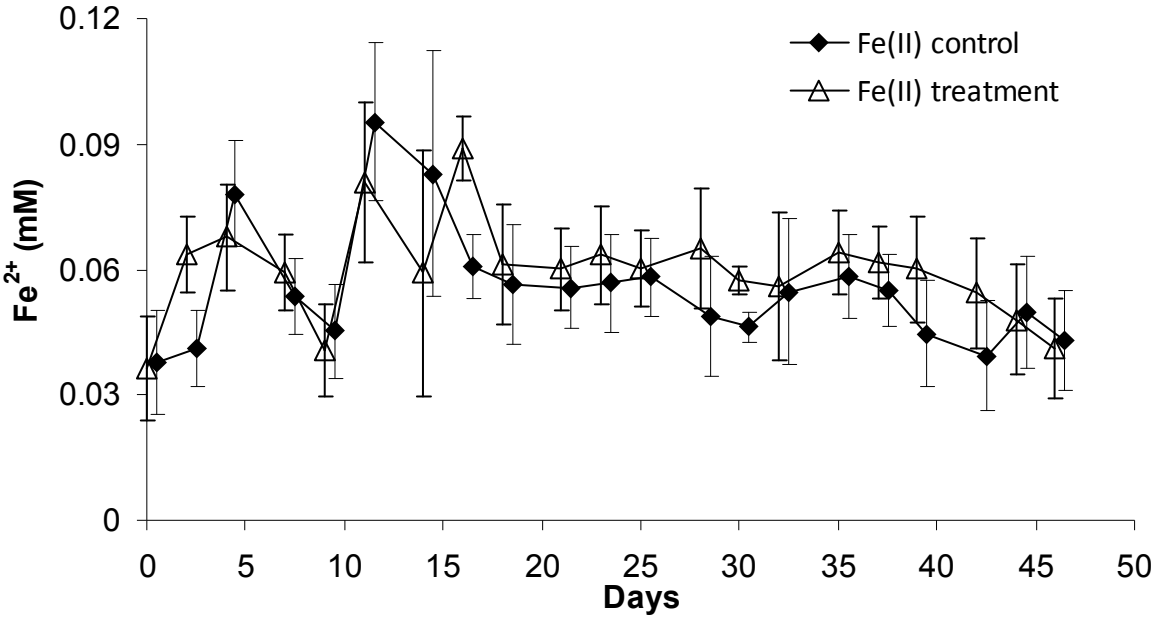

Figure S1. Fe^{2+} in control and Hg amended microcosms (n=3 each) containing Fen sediment (± ISD).

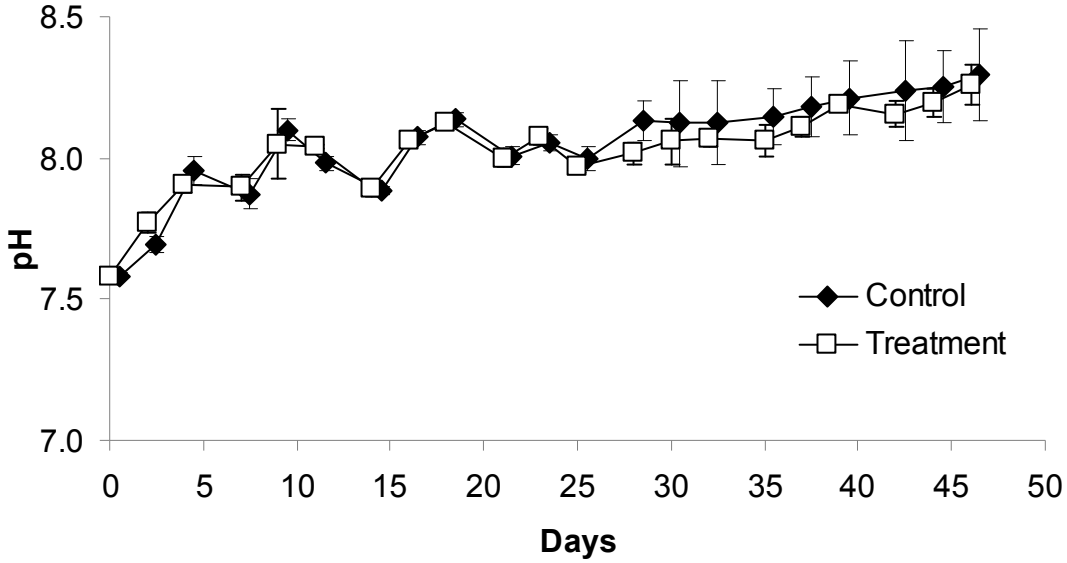

Figure S2. pH in control and Hg amended microcosms (n=3 each) containing Fen sediment (± ISD).

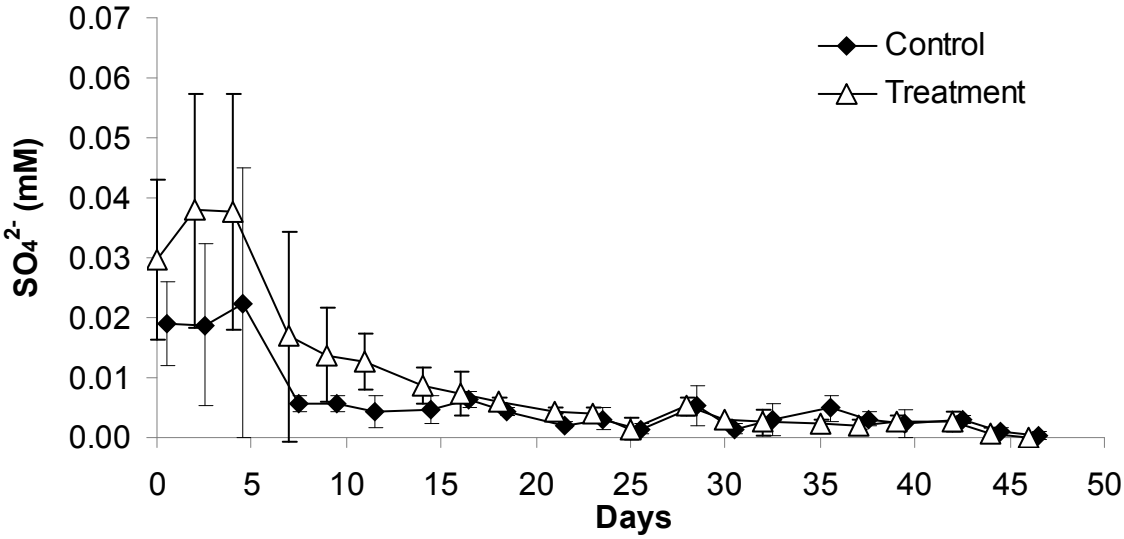

Figure S3. Sulfate concentration in control and Hg amended microcosms (n=3 each) containing Fen sediment (± ISD).

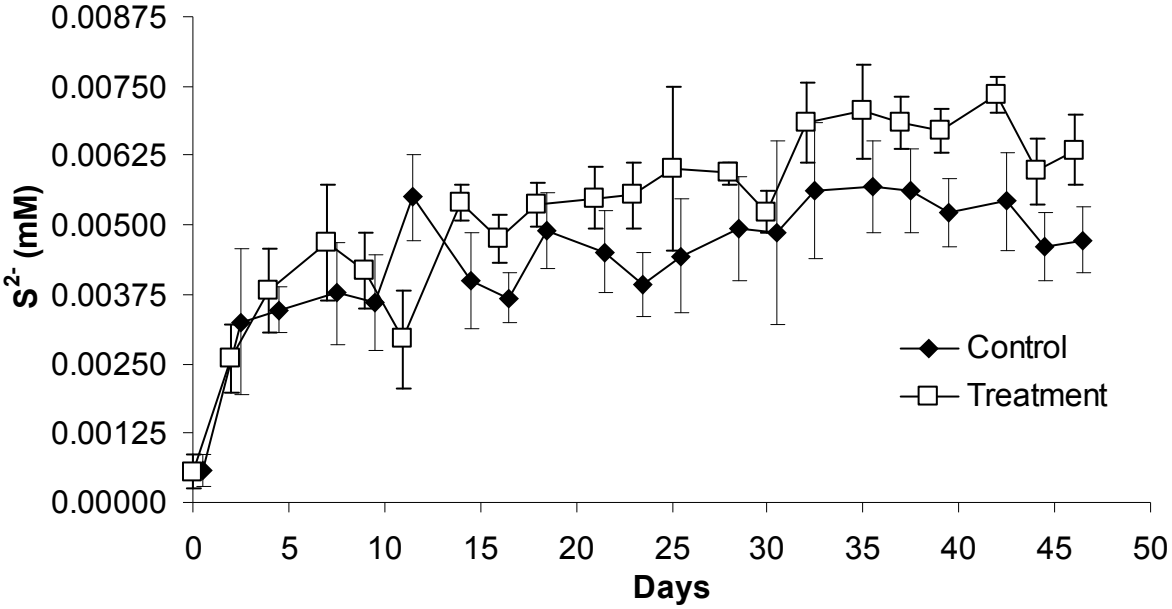

Figure S4. Total sulfide concentration in control and Hg amended microcosms (n=3 each) containing Fen sediment (± ISD).

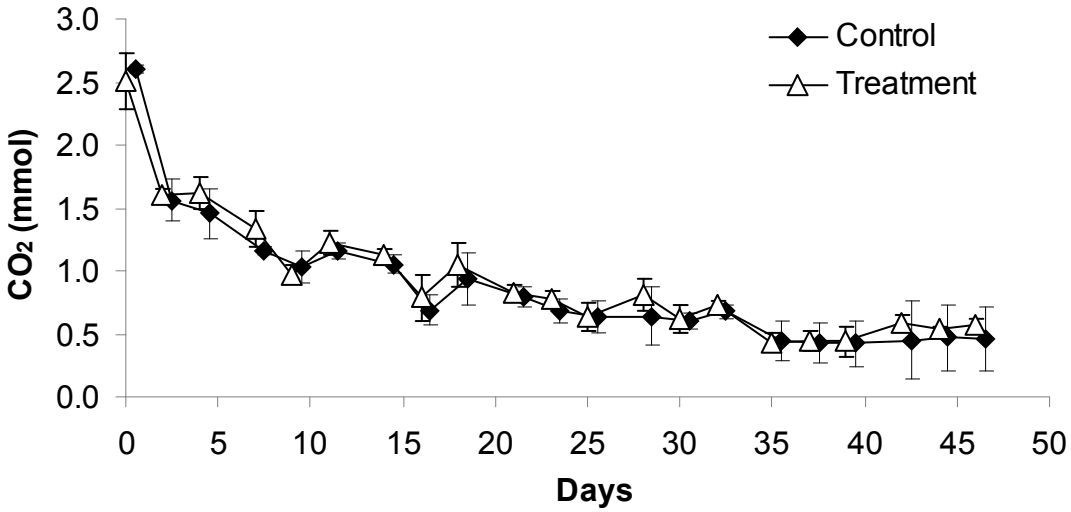

Figure S5. Dissolved carbon dioxide concentration in control and Hg amended microcosms (n=3 each) containing Fen sediment (± ISD).

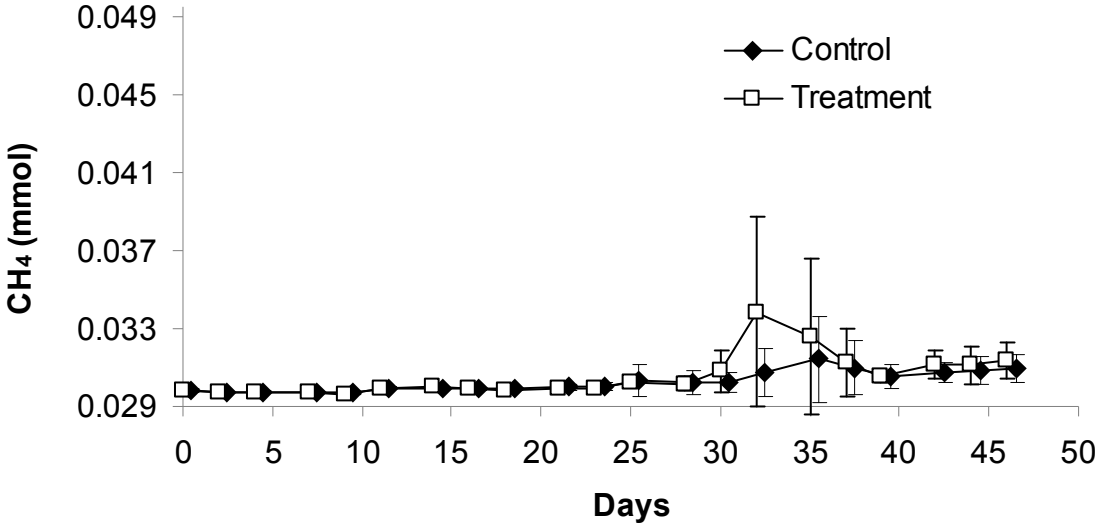

Figure S6. Dissolved methane concentration in control and Hg amended microcosms (n=3 each) containing Fen sediment (± ISD).

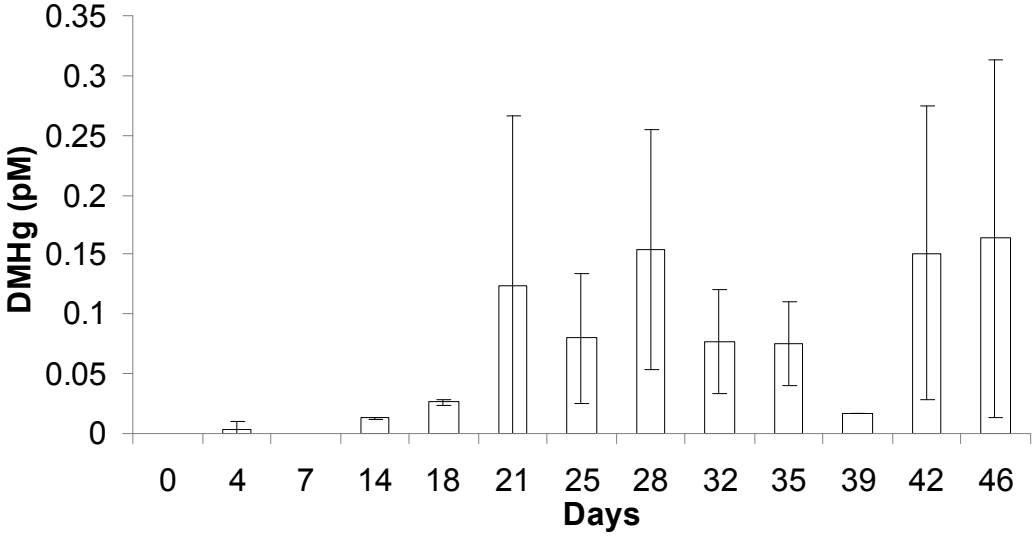

Figure S7. DMHg production, in pM, in control microcosms (n=3) containing Fen sediment (± ISD).

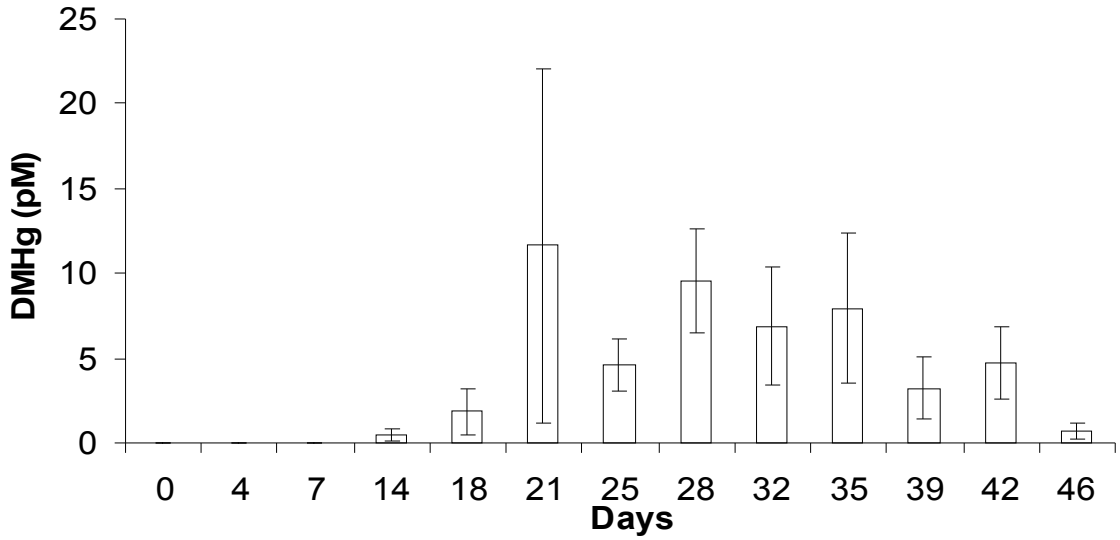

Figure S8. DMHg production, in pM, in treatment microcosms (n=3) containing Fen sediment and amended Hg (± ISD).

Autoclaved Sediment Study

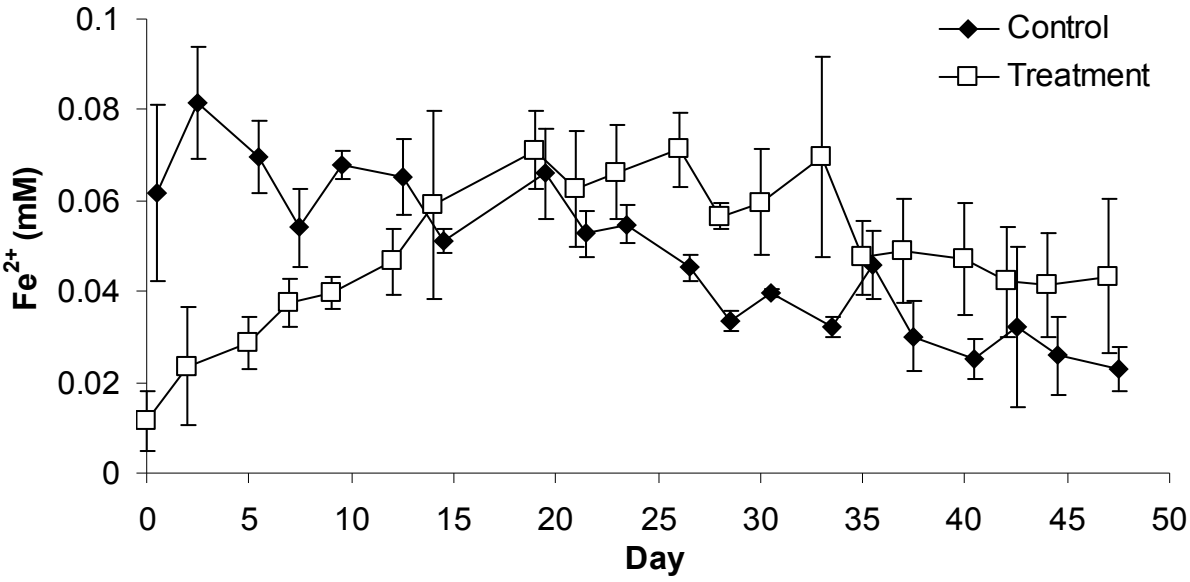

Figure S9. Fe2+ concentration in control (non-autoclaved) and treatment (autoclaved sediment) microcosms (n=3 each) containing Fen sediment (± ISD).

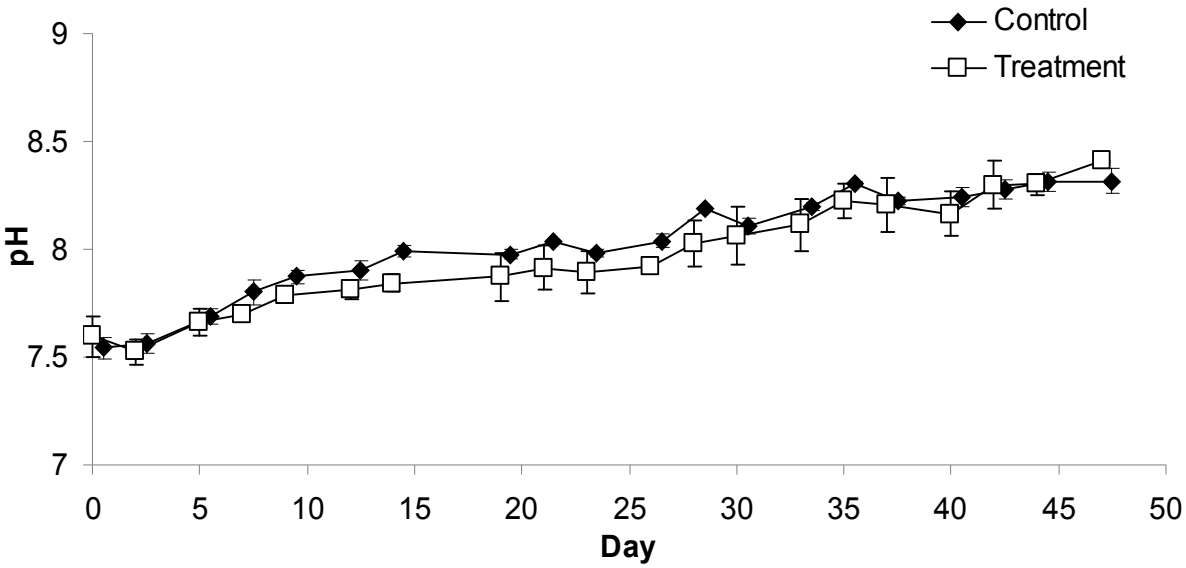

Figure S10. pH in control (non-autoclaved) and treatment (autoclaved sediment) microcosms (n=3 each) containing Fen sediment (± ISD).

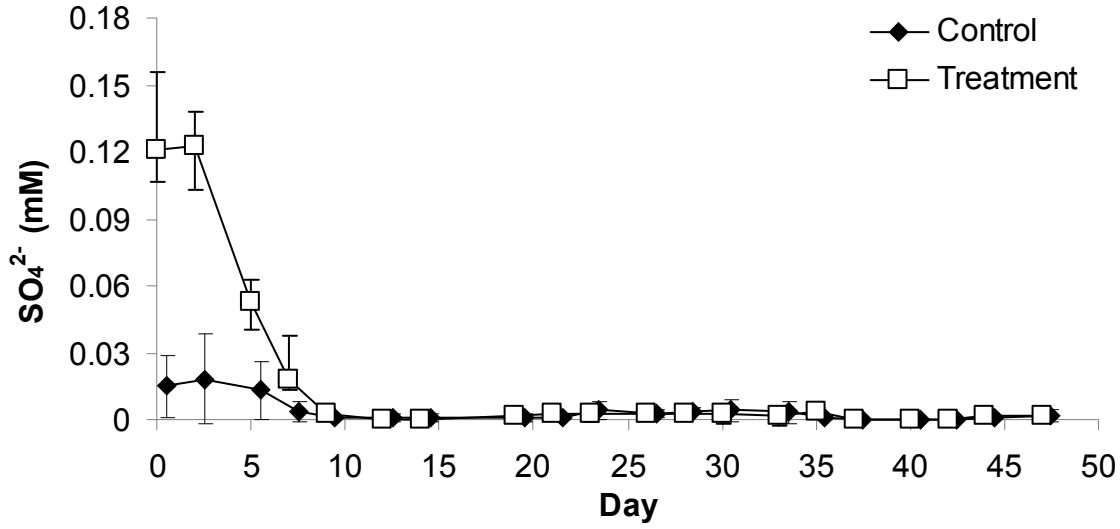

Figure S11. Sulfate concentration in control (non-autoclaved) and treatment (autoclaved sediment) microcosms (n=3 each) containing Fen sediment (± ISD).

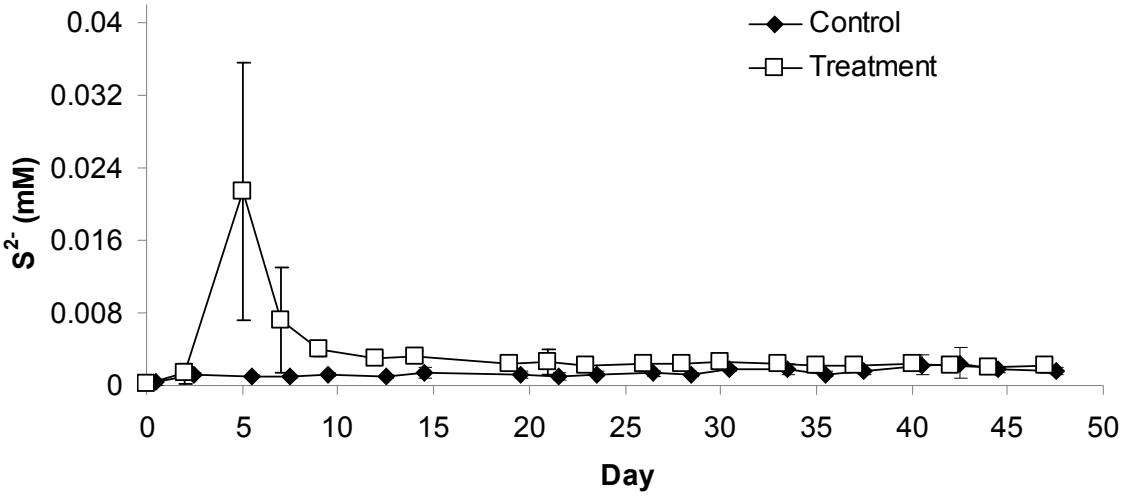

Figure S12. Total sulfide concentration in control (non-autoclaved) and treatment (autoclaved sediment) microcosms (n=3 each) containing Fen sediment (± ISD).

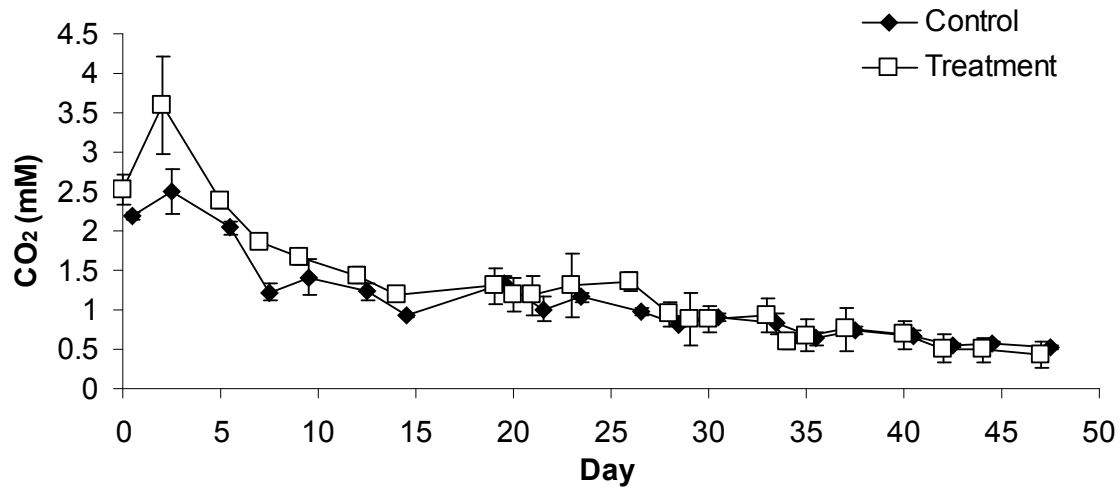

Figure S13. Dissolved carbon dioxide concentration in control (non-autoclaved) and treatment (autoclaved sediment) microcosms (n=3 each) containing Fen sediment (± ISD).

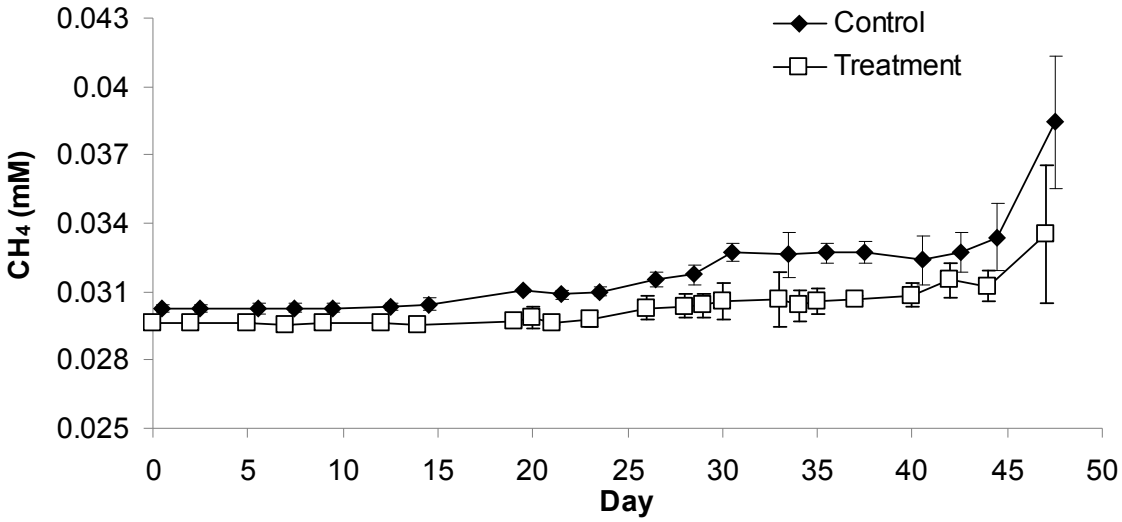

Figure S14. Dissolved methane concentration in control (non-autoclaved) and treatment (autoclaved sediment) microcosms (n=3 each) containing Fen sediment (± ISD).

Autoclaved sediment and acetate addition study

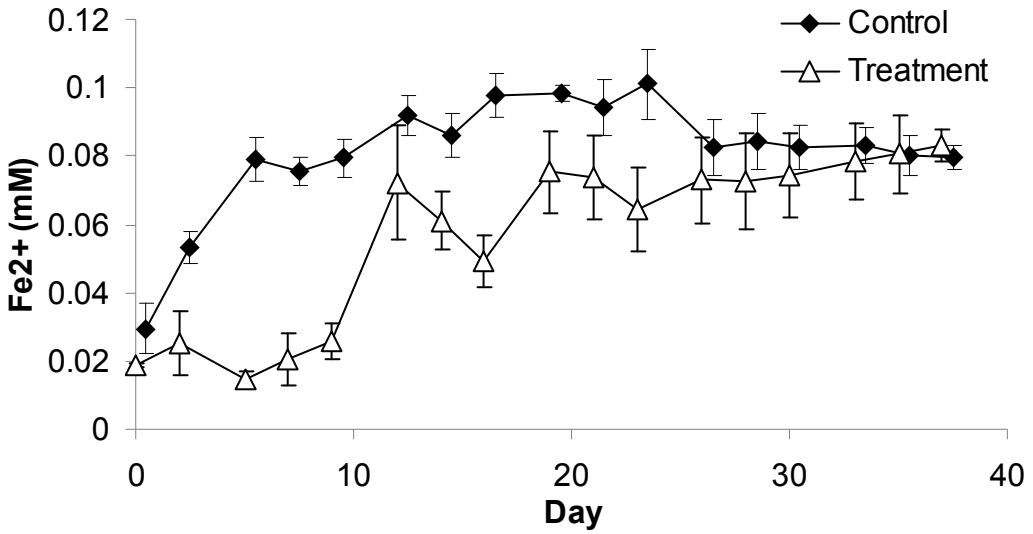

Figure S15. Fe^{2+} concentration in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

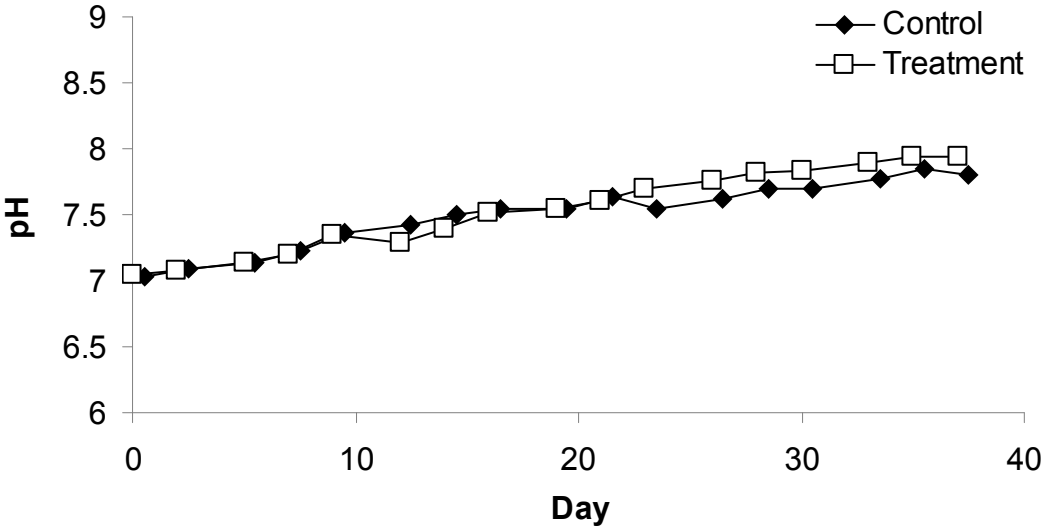

Figure S16. pH in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

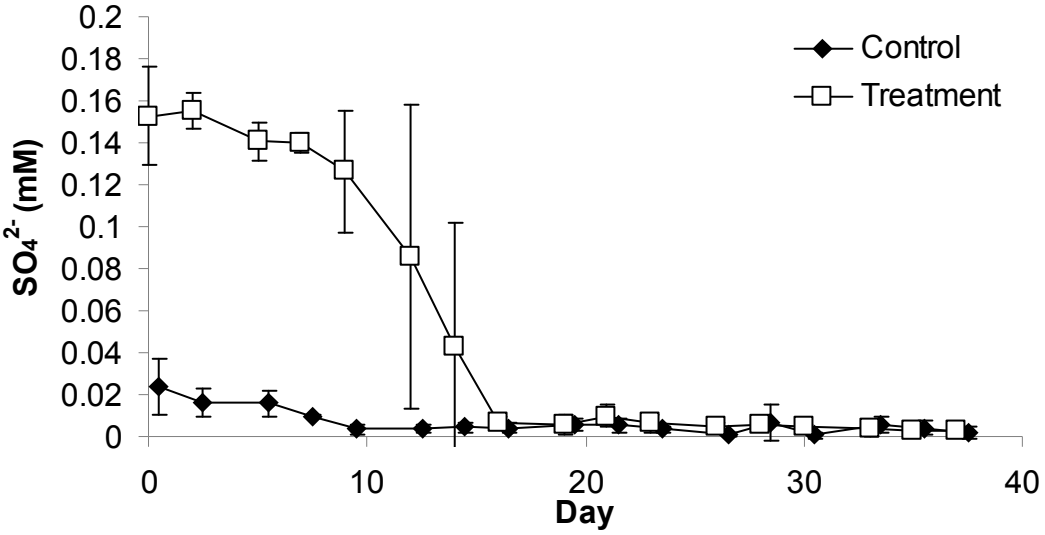

Figure S17. Sulfate concentration in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

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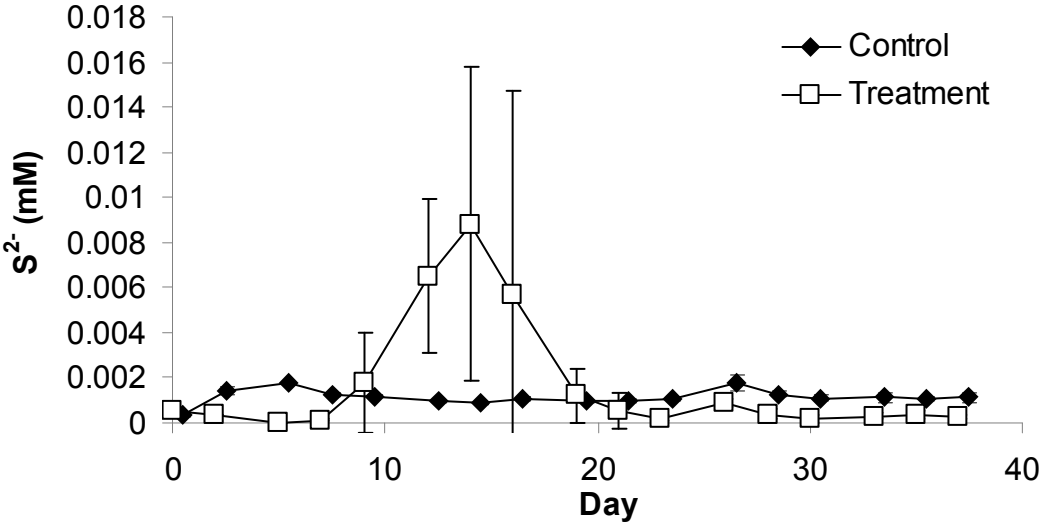

Figure S18. Total sulfide concentration in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

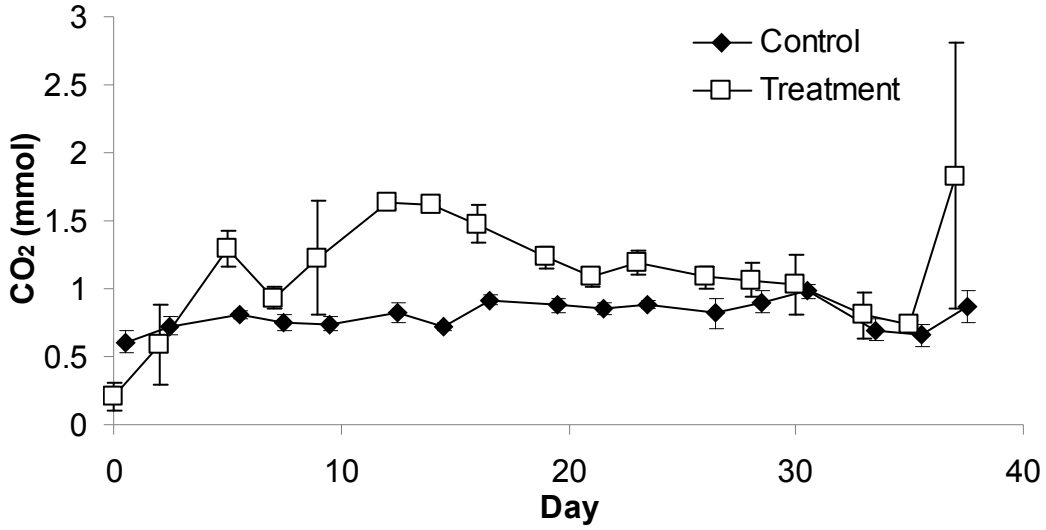

Figure S19. Dissolved carbon dioxide concentration in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

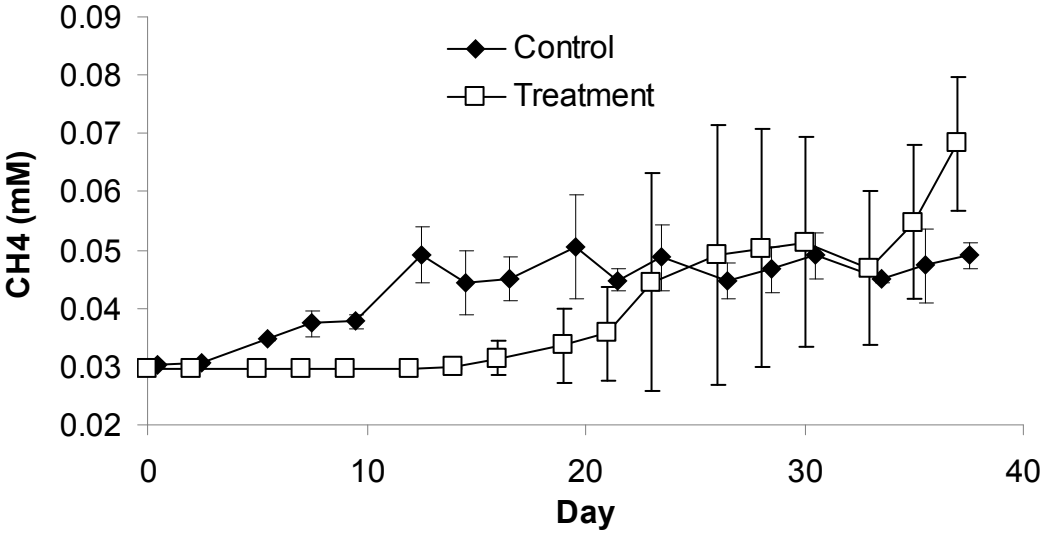

Figure S20. Dissolved methane in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

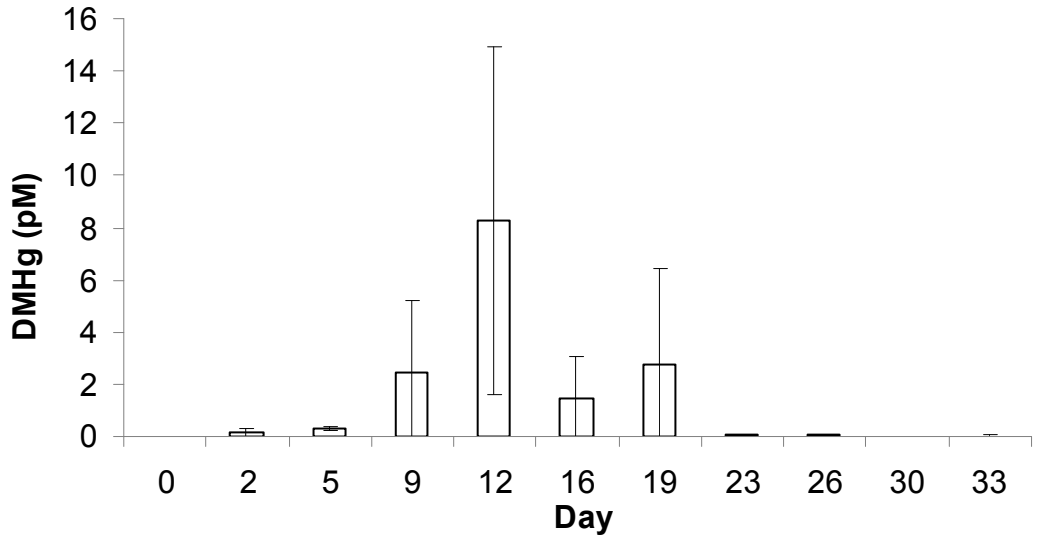

Figure S21. DMHg production, in pM, in control microcosms (n=3) containing Fen sediment and acetate (± ISD).

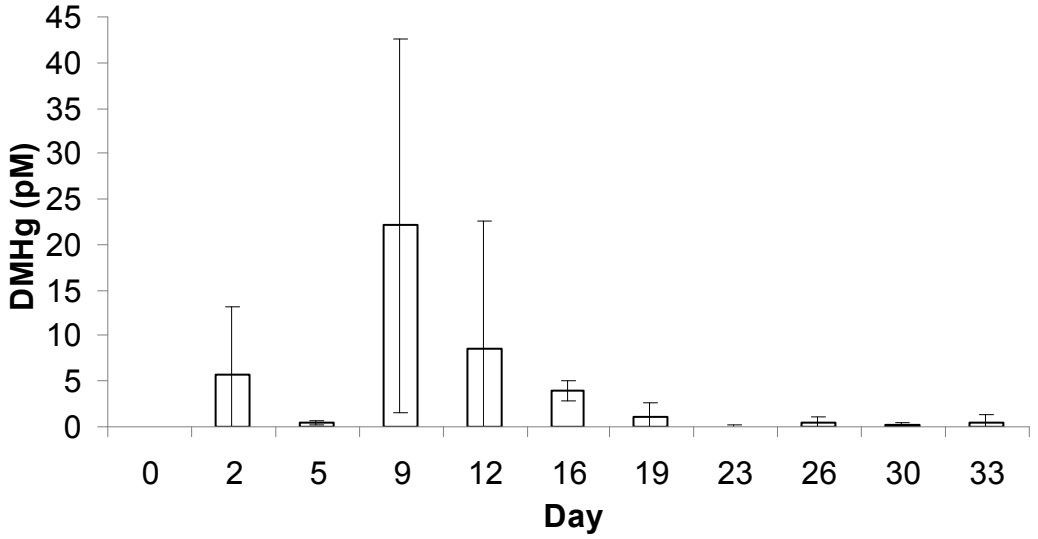

Figure S22. DMHg production, in pM, in control microcosms (n=3) containing Fen sediment and acetate (± ISD).

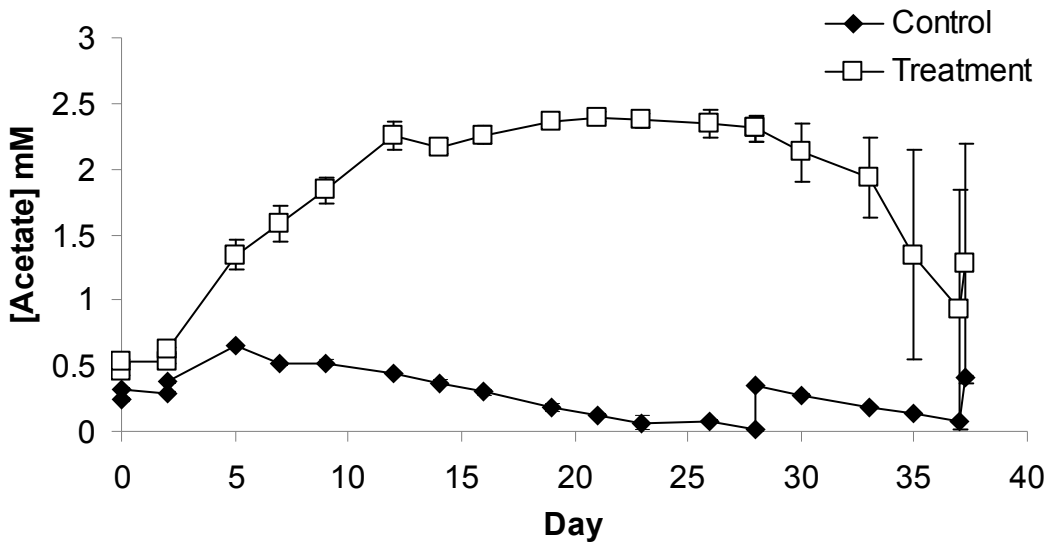

Figure S23. Acetate concentration in control and autoclaved (treatment) microcosms (n=3 each) containing Fen sediment and acetate (± ISD).

APPENDICES

Procedures

Appendix I: SOP for preparation of Deoxygenated Basal Medium

Required Amounts of Reagents:

1. *Mineral Solution or Solution A*: Dissolve the following reagents in 1 liter of filtered DI water in a clean volumetric flask: 4 g of NaCl, 0.5 g of $MgCl_2.6H_2O$, 0.2 mg of $CaCl_2.2H_2O$

2. *Basal Medium:* Dissolve the following in 1 liter of DI water in a clean volumetric flask: 1.5 g of Na_2CO_3 and 200 ml of mineral solution

3. Basal medium can be prepared in advance and stored in a 1L media bottle in the refrigerator.

4. Deoxygenation procedure needs to be repeated before each use.

    a. The solution is purged with nitrogen gas for two hours in order to strip the solution of dissolved oxygen.
    b. Complete deoxygenation is ensured by leaving the solution in an open container for several hours in an anaerobic glovebox.

5. 600mL of the basal media solution is used for each microcosm after adjusting the pH of the solution to ~7 by addition of HCl.

Appendix II: Sediment grab sampling and preparation from the field

1. Sediment is taken from the subsurface with a shovel, from a depth below the root zone, and placed immediately in a container that is then capped and sealed with parafilm.
2. The sediment is then brought back to the lab and placed inside a freezer at -15°C
3. For experiments, the soil was taken into the glove box where 100cc of it is measured out in a 150mL beaker for use in the microcosm experiments. Care is taken not to include any particles greater than $1/8^{th}$ inch (i.e. twigs, roots, etc.)
4. Sediment that is not used is stored back in the freezer.

Appendix III: Microcosm assembly.

1. Two 1/8th inch OD male threaded adapter fittings are inserted into a size 11 silicon cap that has had two holes drilled in it. One adapter is for the sampling port and the other is used as the purging port for collection of DMHg.
2. 1/8th inch teflon tubing is inserted through each of the adapters.
    a. The sampling port tubing is connected onto a two-way valve so that sample may be removed from the microcosm but so it may also remain sealed in between sampling events. This tubing reaches ~1 inch below the surface of the microcosm water.
    b. The purging port tubing is connected to a 20:30 mesh TenaxTrap for collection of DMHg, this tube only goes ~0.5 inches below the silicon cap.
3. The cap with the two ports is placed on a 1L pyrex titration flask that contains the 100cc of sediment, 600mL of basal media, stir bar and added Hg (amount may vary depending on the experiment being conducted). The cap is taped securely on top of the Pyrex flask.
4. Aluminum foil is used to completely cover the microcosm so that it is not exposed to any light for the duration of the experiment.

The microcosm cap was a size 11 silicon stopper (Cole Parmer) that contained two ports: one for sampling of dissolved chemicals and another to capture DMHg during purging. The sampling/purging cap consisted of FEP, PTFE and silicon connective tubing (Cole Parmer), four PVDF fittings (Cole Parmer; two 1/4th to 1/8th inch reducing union and two 1/8th to 1/8th inch pipe adapters), a polycarbonate stopcock for sampling(Cole Parmer), a soda lime trap to trap excess water vapor during purging, and a DMHg trap. The DMHg trap contained 20:30 mesh Tenax (Fisher) which absorbs DMHg for future analysis.

Appendix IV: SOP for Sulfate/Nitrate/Acetate Analysis by Ion Chromatography

Setup

1. While the pump is off and disconnected make sure the containers of DI water located on top of the IC are full. If the containers are not full then they should be taken down and filled with filtered DI water, which is obtained from a Barnstead NANOpure Infinity Ultrapure Water System with a resistivity of 18.0-18.1 MΩ cm. This should be done while wearing gloves so that the water is not contaminated in any way.

2. Once the water is filled and the caps are put back on securely then the IC should be primed to remove any air bubbles that may have gotten trapped in the tubes between runs. This is done by opening the door below the pump and turning the upper left knob counter clockwise until a clicking noise is heard (approx. 2-3 turns). Once the valve is open hit the prime button and let the machine prime for 5 (if the IC has been run recently) to 10 (if the IC has not been run recently) minutes. When it is finished priming hit the off button and close the valve by turning the knob clockwise until it is finger-tight.

3. Open up the correct panel for analysis by clicking on the Browse button, the third from the left, causing a window to pop up. On the left scroll down menu click on the Panels folder and that will bring up a few selections in the right scroll down menu. Double click on either the cations or anions panel, whichever is to be analyzed. The panel will open. On the panel connect the pump, eluent, and suppressor by checking the box to the left of each heading, and then turn the pump on.

4. After the pump is on, set the temperature, eluent, flow rate, and suppressor conductivity values according to which ions are to be sampled. Also make sure the type of eluent is correct for the ions that are being analyzed, MSA for cations and OH for anions. Set the suppressor to CSRS_4mm when analyzing for cations and ASRS_4mm when analyzing for anions. That information is found on the Quality Assurance Reports found in the drawer below the IC. There is a report for both anions and cations.
    - The following values are used to analyze for the anions of interest:
    - The eluent generator is set to 27.5mM (30 for acetate).
    - The oven temperature is set to 35 degrees Celsius (30 for acetate).
    - The conductivity detector is set to 112mA (same for acetate).

5. In order to see how the IC is stabilizing go to the toolbar and click on Control. In the drag down menu click on Acquisition On. There is a window that pops up and shows ECD-1 and ECD-total. When that window pops up click on OK. Once that has been done the baseline should appear on the screen. When the line is flat and the conductivity reading on the IC itself is close to or below 1 then the IC is stable and can be run.

Sulfate/Nitrate/Acetate Standard Procedure

1. The sulfate and nitrate stock solutions (1000mg/L) need to be diluted to 50 mg/L.
2. This is done by filling a 50mL centrifuge tube filled with filtered DI water and then removing 2.5mL of water and replacing it with 2.5mL of the stock solution.

$$C1V1=C2V2$$
$$(1000mg/L)(V1) = (50mg/L)(.050L)$$
$$So, V1 = 2.5mL$$

3. Dilutions of this stock solution must be made for standards with concentrations covering the range of interest, typically 0 – 20mg/L
    a. Example: For a concentration of 2.5 ppm:
        i. (50mg/L)(V1) = (2.5mg/L)(.010L) V1= 0.0005 L
4. So for each standard, the V1 amount of water will be removed from each 10mL tube and replaced with the same amount of 50mg/L standard.
5. ~0.6mL of the standard from each 10mL tube is placed in three 0.5mL Polyvials (Dionex).
6. Acetate standards are made in a similar way, except by dissolving sodium acetate in 100mL of H20, to make a concentration gradient from 0-50mg/L

Sulfate, Nitrate and Acetate Sampling Procedure

1. 5mL glass syringe is inserted into the sampling port of the microcosm. The two-way valve is then opened and sample is removed from the microcosm, the valve is then closed and ~0.6 mL is injected into three separate Polyvials after being filtered by a 0.2 micron filter.

2. A Polyvial filter cap is then pushed down the center of the vial and the vials are placed in an auto-sampler tray which is then placed in the IC auto-sampler.

Appendix V: SOP for Iron analysis by Spectrophotometry

Setup

1. The spectrophotometer is turned on and allowed to warm up for 5 minutes.
2. The Lambda 45 program is opened and the most recent iron method is selected.
3. In the program, the sample info is entered under the sample info menu before each analysis.
4. The spec is blanked, and is then ready for sample analysis.

Preparation of Reagents

10% hydroxylamine solution and 1,10-Phenanthroline solution:

1. The 10% hydroxylamine solution is prepared by dissolving 10g of $NH_2OH \cdot HCl$ in 100mL of filtered DI water. The solution is vortexed to ensure dissolution of the reagent.
2. The 1,10-phenanthroline solution is prepared by dissolving 100mg of 1,10-phenanthroline monohydrate in 100mL of water. 2 drops of 12N HCl solution is then added and the solution is vortexed to ensure dissolution and mixing of reagents.

Iron Standard Procedure

1. The iron stock solution (1000mg/L) needs to be diluted to 50 mg/L.
2. This is done by filling a 50mL centrifuge tube filled with filtered DI water and then removing 2.5mL of water and replacing it with 2.5mL of the stock solution.
3. $$C1V1=C2V2$$
   $$(1000mg/L)(V1) = (50mg/L)(.050L)$$
   $$So, V1 = 2.5mL$$
4. Dilutions of this new stock solution will be made for a concentration range of 0, 1.5, 3, 4.5, 9, 18 and 27 ppm following the procedure above. For a 0 ppm standard, no water will be removed and no standard solution will be added.
   a. For a concentration of 1.5 ppm:
      i. (50mg/L)(V1) = (1.5mg/L)(.010L) V1= 0.0003 L
   b. For a concentration of 3 ppm:
      i. (50mg/L)(V1) = (3mg/L)(.010L) V1= 0.0006 L
   c. For a concentration of 4.5 ppm:
      i. (50mg/L)(V1) = (4.5mg/L)(.010L) V1= 0.0009 L
   d. For a concentration of 9 ppm:
      i. (50mg/L)(V1) = (9mg/L)(.01L) V1= 0.0018 L
   e. For a concentration of 18 ppm:
      i. (50mg/L)(V1) = (18mg/L)(.01L) V1= 0.0036 L
   f. For a concentration of 27 ppm:

i. (50mg/L)(V1) = (27mg/L)(.01L) V1= .0054 L

5. These concentrations are higher than needed, because for sampling 1mL of standard will be used with 2mL of reagent. The final concentrations of iron in the standards analyzed will be: 0, .5, 1, 1.5, 3, 6 and 9 ppm, respectively.
6. So for each standard, the V1 amount of water will be removed from each 10mL tube and replaced with the same amount of 50mg/L standard.
7. Each standard is analyzed by removing 1mL and adding 1mL of acetate buffer, 0.5mL of phenanthroline, and 0.5mL hydroxylamine.
8. Each standard will be vortexed briefly to ensure good mixing.
9. Standard will be analyzed by spectrophotometer at 510nm after waiting 10 minutes for full color development.

Iron Sampling Procedure

1. 100 microliters of 12N HCl is added to a centrifuge tube. This is done to stabilize the soluble Fe(II) before analysis.

2. A 5mL glass syringe is inserted into the sampling port of the microcosm. The two-way valve is then opened and sample is removed from the microcosm, the valve is then closed and 1mL is injected into 15mL centrifuge tubes after being passed through a 0.2 micron filter.

    a. If dilution is necessary, 9mL of filtered DI water is added and the mixture and is then lightly shaken to ensure mixing.

3. 1mL is removed from the centrifuge tube for total and ferrous iron analysis, respectively, and placed in a new centrifuge tube.

4. *For total iron*: 1mL of acetate buffer, 0.5mL of hydroxylamine solution and 0.5mL of 1,10-Phenanthroline solution is added to the 1mL sample.

5. *For ferrous iron*: 1mL of acetate buffer solution and 1mL of 1,10-Phenanthroline is added to the 1mL sample.

6. The solutions are allowed to react for 10 minutes after they are vortexed to ensure good mixing.

7. The 3mL of solution is then analyzed using the spectrophotometer at 510nm.

Appendix VI: SOP for Total Sulfide Analysis by Spectrophotometry

Setup

1. The spectrophotometer is turned on and allowed to warm up for 5 minutes.
2. The Lambda 45 program is opened and the most recent sulfide method is selected.
3. In the program, the sample info is entered under the sample info menu before each analysis.
4. The spec is blanked, and is then ready for sample analysis.

Preparation of Reagents

1. Mixed diamine reagent (MDR)

a. 330mL of concentrated sulfuric acid is added to 170mL of filtered DI water in an ice bath.

b. 1.125g of N,N-dimethyl-p-phenylenediamine oxalate is dissolved in the sulfuric acid solution after it has cooled. This may take several hours.

c. In a 100mL volumetric flask, 2.7g of ferric chloride hexahydrate is dissolved in 50mL of concentrated hydrochloric acid which is then diluted to 100mL.

d. This solution is stable indefinitely.

2. Sulfide stock solution (~50 micromoles/mL sulfide)

a. 50mL of deoxygenated filtered DI water is added to a 60mL serum bottle.

b. On a paper towel, a few crystals of sodium sulfide ($Na_2S \cdot 9H_2O$) are rinsed with filtered DI water to remove the oxidized surface layer.

c. The crystals are patted dry and ~0.6g are weighed out and placed in the 60mL serum bottle.

d. The sodium sulfide will dissolve slowly, and the solution must be stored in a cool, dark location.

e. Weekly standards are make from a dilution of this stock solution (0.5 micromoles/mL)

Standardization of the stock solution

1. Reagents

a. 0.025N standard iodine solution.

b. 0.025N standard thiosulfate solution.

c. Starch indicator; dissolve 1g of soluble starch indicator (Fisher) in 100mL of boiling filtered DI water.

2. Method
    a. 10mL of 0.025N standard iodine solution is added to two 125mL Erlenmeyer flasks.
    b. 5mL of deoxygenated 6M HCl is then added to each flask.
    c. 1mL of the 0.5 µmol/mL stock solution is added to one flask while 1mL of filtered DI water is added to the other.
    d. Each flask is swirled once by hand, covered with parafilm and then covered in aluminum foil for five minutes.
3. e. After five minutes, 1mL of soluble starch indicator is added to each solution and then they are titrated with 0.025N thiosulfate solution. The endpoint is reached when the blue color disappears and the solution becomes clear or white from a dark blue.
    e. Concentration calculation:

concentration of sulfide (µmol/mL) = (volume of titrant used in the blank – volume used in the sample) x 12.5

Sulfide standard preparation

1. ***The 0.5*** µmol/mL stock solution is used to prepare standards over the concentration range of 0 – 1mg/L with a volume of 2mL.
2. 0.2mL of mixed diamine reagent are added to the 2mL of standard and the solution is allowed to react for 30min before analysis by spectrophotometer at 670nm.

Sulfide sampling procedure

1. A 5mL glass syringe is inserted into the sampling port of the microcosm. The two-way valve is then opened and sample is removed from the microcosm, the valve is then closed and 2mL is injected into a 15mL centrifuge tube after being passed through a 0.2 micron filter.
2. 0.2mL of mixed diamine reagent is added and the solution is allowed to react for 30min before analysis by spectrophotometry at 670nm.

Appendix VII: SOP for Mercury Analysis by Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS)

Standard Preparation

1. One hour prior to analysis the Ar gas flow at the CVAF detector is turned up to 90 mL min^{-1}.
2. Variacs 2 and 3 on the detector are turned on.
3. A bottle of 1% NaTEB solution from the freezer is allowed to thaw.
4. A new soda lime trap is prepared and attached to the bubblers and gas lines.
5. The sample traps are blanked by heating for 2 min each with the injection valve in the horizontal "inject" position.
6. DMHg analysis:
    a. Blanks for the bubbler are ran at the beginning of analysis and periodically throughout the analysis.
    b. The bubblers are rinsed with MilliQ, and filled with 200 mL MilliQ water.
    c. 0.4 mL of sodium acetate buffer and 0.2 mL of 1% NaTEB solution is added.
    d. The bubbler is capped and swirled to homogenize the solution. The solution is allowed to react for 2–4 min without sparging. The injection valve is in the vertical "load" position during this time.
    e. The three-way stopcock is rotated to divert N_2 flow from the headspace of the bubbler to the sparging stem. The solution is then purged for 12 min.
    f. After sparging, rotate the three-way stopcock so that N_2 flow is through the headspace; allow the Tenax traps to "dry" in this position for 5 min.
    g. After drying the injection valve is turned to the horizontal position and the gas flow is allowed to equilibrate for 2 min.
    h. The bubbler is now closed off from the analytical system, which is then opened and drained and rinsed with MilliQ.
    i. After 2 min in the "inject" position, the analytical sequence on the Crontrol is started for sample analysis:
    j. For Bubbler 1 (left side, top Tenax trap) press: [TIME] [0] [CIRCUIT] [2] [ON], and press [START] on the integrator.
    k. For Bubbler 2 (right side, bottom Tenax trap) press: [TIME] [100] [CIRCUIT] [3] [ON], and press [START] on the integrator.
    l. The peak area (PA) of the dimethylmercury is recorded (~1 min.).

Appendix VIII: SOP for pH meter

Standard preparation

1. Three standards of known pH (4, 7 and 10) are obtained.
2. The pH electrode is rinsed with filtered DI water and inserted into the 4pH standard. Once the pH level stabilizes the value is recorded and the 'standardize' button on the pH meter is pressed.
3. The pH electrode is rinsed with filtered DI water before being inserted into another standard (pH 7 and 10) or sample.

pH sampling procedure

1. A 5mL glass syringe is inserted into the sampling port of the microcosm. The two-way valve is then opened and sample is removed from the microcosm, the valve is then closed and 2.5mL is injected into a 15mL centrifuge tube.
2. The pH meter is then placed inside the centrifuge tube and the reading is recorded.

Appendix IX: SOP for GC Analyses

6890 Procedure

1. The gas tanks will be checked (Helium, Hydrogen, Nitrogen and Air) for appropriate pressures. (Appropriate levels are marked on the regulators- Tank pressure should never read below 500 psi and line pressure should never exceed recommended limit marked on the gauge)
2. The head space injection nut will be unscrewed and the septa replaced. The screw will then be screwed back on finger-tight.
3. The 'PREP.M" method from the method pull down method will be selected on the GC computer screen. Prep. Run will take 30 min.
4. Once Prep.Run is complete, the 'NEW DUAL.M" method will be selected from the pull down menu.
5. After approximately 10 min, the GC will be ready for analysis with 'Ready' sign in green.
6. The user information including file name (under which all results will be saved) with date, operator/user name and sample name will be entered under the 'RunControl' and then 'Sample info' tab on the GC screen.
7. For gaseous samples, 50 µl of the sample gas will be injected directly into the GC through the head space injection nut by using a Hamilton glass gas-tight syringe. The start button will be hit immediately on the GC and after approximately 22 seconds, the syringe will be removed.
8. Once the run is complete, GC will show 'Not Ready' sign in red and the next sample will be ready to be injected after the computer shows the 'Ready' in green.
9. The chromatographs will be viewed by going to 'Method' and 'Run Control' pull down menu and selecting data analysis.
10. After analysis is complete, the computer will be shut down by going to the 'Method' pull down menu and selecting "Shut_OGC.M".

5890 Procedure

1. The gas tanks will be checked (Helium, Hydrogen, Nitrogen and Air) for appropriate pressures. (Appropriate levels are marked on the regulators- Tank pressure should never read below 500 psi and line pressure should never exceed recommended limit marked on the gauge)
2. The head space injection nut will be unscrewed and the septa replaced. The screw will then be screwed back on finger-tight.

3. The 'PGAS_COND.M" method from the method pull down is selected on the GC computer screen. This is the conditioning method that will prepare the machine for analysis and will run for 30min.
4. After the machine has conditioned, the 'PGAS_SP2.M' method from the method pull down is selected and ran. This prepares the GC for oxygen and carbon dioxide analysis.
5. The user information including file name (under which all results will be saved) with date, operator/user name and sample name will be entered under the 'RunControl' and then 'Sample info' tab on the GC screen.
6. For gaseous samples, 50 µl of the sample gas will be injected directly into the GC through the head space injection nut by using a Hamilton glass gas-tight syringe. The start button will be hit immediately on the GC and after approximately 10 seconds, the syringe will be removed.
7. After analysis is complete, the computer will be shut down by going to the 'Method' pull down menu and selecting "SHUT5890.M".

Sampling Procedure for Dissolved Gases

1. 4.5mL of sample is removed from the microcosm with a syringe and injected into a glass vial with an internal volume of 9mL. The vial is capped and sealed with a Teflon cap and aluminum seal.

2. After the sample is allowed to equilibrate, a 50microliter sample of the head space from the vial is obtained and injected into the GC. The needle is left inside the injection port until 10s on the 5890 and 0.22min on the 6890.

3. Methane peaks arrive at ~1.5min on the 6890, Oxygen at ~1.3min and CO2 arrives at ~ 5.9min on the 5890.

4. Concentration of gases is calculated from the calibration data which involves the peak area information on the read out sheet.

Appendix X: SOP for Dissolved Methane Analysis by Gas Chromatography

Standard Preparation

1. Seven 9 ml glass bottles were filled with 50% DI water (9ml) and capped and crimped.

2. Each bottle was filled with different volumes of methane: 0, 10, 25, 75, 100 and 250 µl.

3. For each volume injected the concentration was determined as follows:
   a. First the number of moles, n, was determined using the ideal gas law;

$$n = \frac{PV}{RT}$$

   where R = gas constant (0.0821 atmL/molK; T = temperature (298K); P = Pressure (1 atm); V = volume of gas injected.

   b. The number of moles was divided by the aqueous volume (Vw) to give moles per liter before partitioning and then the moles per liter were converted to the concentration (C_w) in mg per liter using the molecular weight of methane (16 grams/mole):

$$C_w = \frac{n}{V_w} x\, 16\, \frac{g}{mole}$$

   c. The total mass was determined by:

$$m = C_w * V_w$$

   where, V_w = volume of water.

   d. A dimensionless Henry's constant (K_H') of 28.5 for Methane at 20°C was used to determine the fraction in water (f_w):

$$f_w = \frac{1}{\left(1 + K_H'\left(\frac{V_a}{V_w}\right)\right)}$$

   where, V_a = volume of air.

   e. The fraction in water was used to determine the mass in the aqueous phase after partitioning (m_w):

$$m_w = f_w\, x\, m$$

   f. And the mass in head space (m_a)

$$m_a = m\, x\, (1 - f_w).$$

g. The concentrations in the aqueous phase (C'_w) and head space were determined using the respective masses and volumes:

$$C'_w = \frac{m_w}{V_w} \text{ and } C_a = \frac{m_a}{V_a}.$$

4. The bottles were rotated for 1 hr and then analyzed by direct headspace injection (50 µl) on the GC.
5.

Sample Concentrations

1. Using a gas-tight 50µl syringe, a sample was taken from the reactors and injected in the GC to give a peak area.
2. The peak area (PA) was used to calculate the concentration in the headspace by the standard curve equation.
3. The total mass was determined using the headspace volume and the fraction in water:

$$m = \frac{C_a \, x \, V_a}{1 - f_w}$$

4. The dissolved concentration was then determined using the total mass, the aqueous volume and the fraction in water:

$$C'_w = \frac{m * f_w}{V_w}$$

Appendix XI: SOP for Dissolved Oxygen Analysis by Gas Chromatography

Standard Procedure

1. Four different volumes (50, 25, 10 and 5 µl) of 99% pure oxygen are injected into the GC using a 50 µl gas-tight syringe with 50 µl taken as 100% and 1,000,000 ppmv.

2. The peak area was taken as partial pressure of oxygen in the headspace, with 50 µl taken as 1atm, 25 as 0.5atm, 10 as 0.1atm and 5 as 0.05atm.
3. Peak area vs. concentration is then plotted to give the calibration curve.

Sample Calculations

1. Using a gas-tight 50µL syringe, a sample was taken from the sample vial and injected in the GC to give a peak area.

2. The peak area (PA) was used to calculate the partial pressure (Pi) in the headspace in atm by the equation:

$$P_i = 6E^{-6}PA - 0.0024$$

3. The aqueous concentration was calculated using Henry's law and a Henrys constant (k_H) of 1.3x 10^-3 mole/L atm and converted to mg per liter using the molecular weight of oxygen (32 grams/mole) and doing a unit conversion:

$$C'_w = k_H x P_i \; x\, 32 \; \frac{g}{mole} \; x\, 1000 \; \frac{mg}{g}$$

4. The total mass (m) was found by calculating the mass in the aqueous phase using the aqueous volume (V_w) and adding it to the mass in the headspace, which was determined using the ideal gas law calculated with the partial pressure and the volume in the headspace (V_a):

$$m = \left(\frac{P_i V_a}{RT} \; x\, 32 \frac{g}{mole} \; x\, 1000 \frac{mg}{g}\right) + \left([C]'_w \, x \, V_w\right)$$

where R = gas constant (0.0821 atm L/mol K; T = temperature (298K)

Appendix XII: SOP for Dissolved Carbon dioxide analysis by gas chromatography

Carbon Dioxide Calibration Curve

1. Turn the from 50/50% H_2/CO_2 gas tank to open, allowing gas to flow freely from the line.
2. Use a gas tight syringe to directly pull varying amounts of gas from the H_2/CO_2 gas line.
3. Inject the gas sample into the 5890 GC. The run time for CO_2 is approximately six minutes.
4. Each injection volume was done in triplicate.

*applicable to analyses using a 50ul gas tight syringe

CO_2 Standard	Injection Volume (µL)	$\%CO_2$	Partial Pressure of CO_2
1	10	10	0.1
2	20	20	0.2
3	50	50	0.5

Inorganic Carbon Calculation

Mass of Total Inorganic Carbon = Mass of Inorganic Carbon in the Headspace (TIC) + Ma

1. Mass of Inorganic Carbon in the Headspace (moles)

$$PV = nRT \quad \text{or} \quad n = PV/RT$$

where P= partial pressure of Carbon Dioxide from calibration curve,

V= headspace volume

R= Gas Constant=0.082 L*atm/K*mole

T=Temperature= 298.15 K (273.15 + t, t = 25 C)

n= moles of carbon dioxide in headspace (unknown)

2. Inorganic Carbon in the Aqueous Phase

$$Dissolved\ Inorganic\ Carbon(DIC) = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}]$$

$$[H_2CO_3^*] = K_H * pCO_2$$

Where $K_H = 10^{-1.47}$ and pCO_2 is measured

$$[HCO_3^-] = \frac{K_1 * K_H * pCO_2}{[H^+]}$$

Where $K_1 = 10^{-6.35}$, $K_H = 10^{-1.47}$, pCO_2 and $[H^+]$ are measured

$[CO_3^{2-}]$ **is neglible** at/near neutral pH

Mass of Inorganic Carbon (IC) in Aqueous Phase (moles) = DIC (mole/L) * aqueous phase volume (L)